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# P.32 CHROMATOGRAPHIC DETERMINATION OF TOCOPHEROLS, SUGARS AND FATTY ACIDS IN WILD FRUITS

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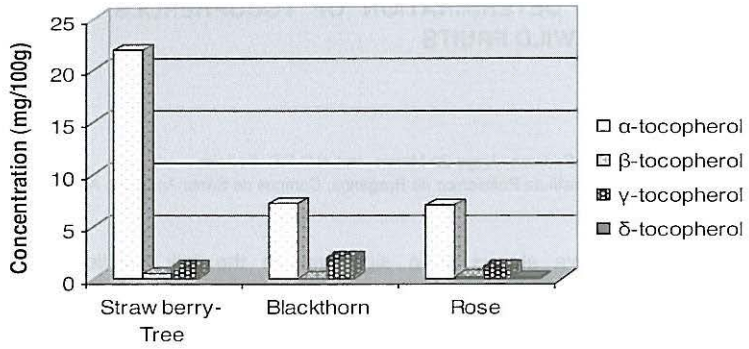
Food and medicinal plants have always been significant in the folk traditions of the Mediterranean area. Similar diets and ailments treated with plants, as well as, related practices in preparation and administration of the folk remedies reflect an important heritage, which constitutes a base for phytochemical and pharmacological studies that can lead to new therapeutic and nutraceutical products[1].

Three wild fruits (strawberry-treeand blackthorn berries and dog rose hips) were analyzed for tocopherols by high performance liquid chromatography (HPLC) coupled to a fluorescence detector, sugars by HPLC coupled to a refraction index detector (RID), and fatty acids by gas-chromatography (GC) coupled to a flame ionization detector (FID).

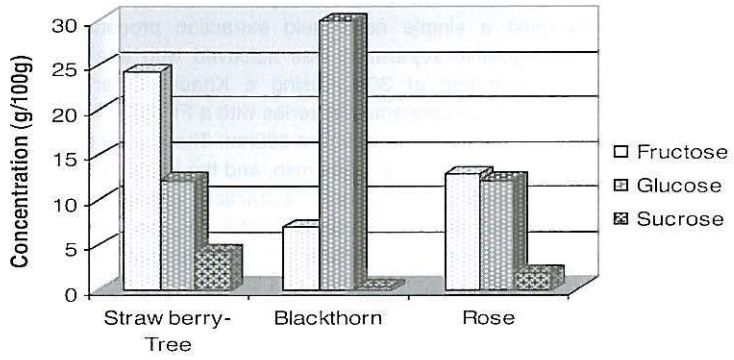
For tocopherols analysis it was used a simple solid-liquid extraction procedure without saponification step and the chromatographic separation was achieved with a a YMC-Pack Polyamine II column (250x4.6mm) operating at 30°C, using a Knauer Smartline HPLC equipment with a 2500 UV detector at 295 nm connected in series with a FP-2020 fluorescence detector programmed for excitation at 290nm and emission at 330nm. The mobile phase used was hexane/ethyl acetate (70:30, v/v) at a flow rate of 1.0mL/min, and the injection volume was 20$\mu$l. For sugars analysis it was used a solid-liquid extraction procedure and the chromatographic separation was achieved with a Eurospher 100-5 $NH_2$ column (4.6mm x 250mm, 5mm) operating at 35°C, using a Knauer Smartline HPLC equipment with RID. The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1mL/min, and the injection volume was 20$\mu$l. The fatty acid profile was analyzed, after a trans-esterification procedure, with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID and a Macherey-Nagel column (30m x 0.32mm ID x 0.25$\mu$m df)[5]. The oven temperature program was as follows: the initial temperature of the column was 50°C, held for 2min, then a 10°C/min ramp to 240°C and held for 11min. The carrier gas (hydrogen) flow-rate was 4.0mL/min (0.61 bar), measured at 50°C. Split injection (1:40) was carried out at 250°C.

The values obtained in the analysis of the samples point to the existence of differences in what concerns tocopherols composition (Figure 1). α-Tocopherol was the major compound in all the fruits, and δ-tocopherol was only detected in blackthorn fruits. Strawberry-tree fruits presented the highest content of tocopherols (23.46mg/100 g of dry weight) while rose fruits revealed the lowest content (8.33mg/100g). α-Tocopherol was the principal form of vitamin E, it is a lipid-soluble antioxidant and it functions as a chain-breaking antioxidant for lipid peroxidation (LP) in cell membranes and also as a scavenger of reactive oxygen species such as singlet oxygen. It is considered to serve as the first line of defence against LP, and it protects PUFAs (polyunsaturated fatty acids) in cell membranes from free radical attack through its scavenging activity in biomembranes at early stages of LP.[2]




**F1** Tocopherols composition (mg/100 g of dry weight) of the wild fruits.

In what concerns sugar composition (Figure 2) the wild fruits presented fructose, glucose and sucrose as main sugars.




**F2** Sugars composition (g/100 g of dry weight) of the wild fruits.

For strawberry-tree (24.21g/100g) and rose fruits (12.89g/100g) fructose was the most abundant sugar, while glucose predominates in blackthorn samples (29.84g/100g). Strawberry-tree fruits revealed the highest total sugars content, and highest levels of fructose and sucrose (Figure 2), which is in agreement with its sweet taste. Otherwise, rose fruits showed the lowest levels in total sugars (26.90g/100g).

The major fatty acids found in strawberry-tree and rose fruits were α-linolenic acid (C18:3) and linoleic acid (C18:2), contributing to the prevalence of PUFA in these samples. In blackthorn fruits, MUFA (monounsaturated fatty acids) predominated over MUFA due to the abundance of oleic acid (C18:1). These fruits also present high levels of linoleic acid but significant lower amounts of α-linolenic acid than the other two wild fruits.

Overall, strawberry-trees revealed the highest contents in sugars and tocopherols while rose fruits showed the highest content in PUFA. This study contributes not only to a better knowledge of these wild fruits but also to their valorisation.

Acknowledgements
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References:

[1] Hadjichambis A. *et al.* International Journal of Food Science and Nutrition, 2008, 59, pp 383-414.
[2] Kanu et al. Trends Food Science Technology, 2007, 18, pp 599-608.